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CONTRIBUTION OF DIETARY AND ENVIRONMENTAL IODINE TO IODINE BALANCE OF RAINBOW TROUT (*SALMO GAIRDNERII*)

by

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A THESIS

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ABSTRACT

This study was designed to discover the relative importance and contribution of dietary and environmental iodine supplies to iodine balance in the rainbow trout, Salmo gairdnerii.

The rate of iodine uptake from the water is influenced by the iodide concentration of the water. Increased concentrations of environmental iodine decrease the rate of iodine uptake from the water, but increase the total quantity of body iodine. Iodine-poor water increases the rate of uptake from the water, but whole body concentrations of iodine remain low. It appears that environmental iodide enters the body by passive diffusion through gill and oral membranes.

The nutritional state of fish influences iodine accumulation, although the effects are less marked than the effects caused by varied environmental iodine concentrations. Although dietary iodide is lost rapidly by fish in iodine-enriched water, decreased fecal volume seems to greatly decrease biliary excretion of iodine. Consequently, in iodine-enriched water, fed fish remain in a steady state, while starved fish, paradoxically, come into positive iodine balance.

Body iodine is distributed throughout different body tissues in much the same proportion as chloride. Iodide is slow to penetrate brain tissue. The thyroid gland, skin and gut concentrate proportionately greater amounts of iodide than chloride.

The thyroid gland appears to have some degree of regulation over the quantity of iodine it contains, but body iodine content varies

passively in accordance with the amount of iodine available. In fed fish, iodine enrichment of the water increases the thyroidal concentration of I^{127} by only 12%, but body concentration increases almost 165%. Similarly, in starved fish, thyroid concentration increases 109%, while body concentration increases by over 530%.

In conclusion, it appears that food, even though rich in iodine, plays a lesser role than water in contributing to the iodine balance of trout, even though water may contain only small amounts of iodine.

ACKNOWLEDGEMENTS.

I wish to express my deepest gratitude to:

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Iodide accumulation is the first essential step leading to the synthesis of thyroid hormone by the thyroid gland of vertebrates. The environment is the source of iodine for all vertebrates, but the form in which iodine is obtained and the pathways through which it may enter the body differ.* Terrestrial vertebrates obtain iodine only from the food and water they ingest. However, fish and other aquatic or amphibious vertebrates may receive iodine from several different sources: ingested food and water, water in contact with the gills and oral membranes, and water in contact with the integument.

It is well known that the relative abundance of iodine in the environment and the rate at which it enters the body influence the iodine-accumulating activity of the thyroid gland. Among fish, the concentration of iodine in the water is particularly important. The enrichment of water with iodine is known to produce histological quiescence of the thyroid and to decrease the uptake of iodine by the thyroid (Gorbman and Bern, 1962). High concentrations of iodine (I^{127} as iodide-iodate) in the sea water reduce the accumulation of radioiodine by the thyroids of marine fish. The effect is complicated by the high salinity. A low concentration of iodine in fresh and salt water increases thyroidal uptake of I^{131} (Hickman, 1959).

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* Throughout this study, the term "environmental iodine" is used to denote iodine, present as inorganic ions, dissolved in the ambient water. Dietary iodine is restricted to mean organic and inorganic iodine present in the food.

A recent comparison by Hickman (1962) of iodine metabolism in a fresh-water fish and a marine fish emphasizes this point. The iodine concentration of fresh water remains stable throughout the year and so does the iodine content of the thyroids of whitefish which inhabit it. The iodine concentration of the estuarine sea water flounder varies seasonally. This is due to fluctuations in the flow of the rivers which drain the coast. Large amounts of fresh water dilute the sea water and decrease the iodine concentration. These periods of low iodine concentration are reflected in the low iodine content of the thyroids of flounder present in these coastal waters (Hickman, 1962).

The diet is also a source of iodine for fishes. Thyroidal iodine contents of pelagic marine fishes vary seasonally. The fluctuations correspond to similar variations in the iodine content of plankton and seaweed. Stomach contents of fish rich in body iodine contain more iodine than do stomach contents of fish poor in body iodine (Vinogradov, 1953). It can be concluded that iodine metabolism is influenced by the availability of both dietary and environmental iodine. However, the relative importance of dietary sources of iodine and that iodine derived directly from the water are unknown. Here, then, lies the objective of this study: to discover and evaluate the contribution of dietary and environmental iodine to iodine balance in rainbow trout.

Ideally, the use of radioisotope techniques, coupled with an analysis of stable iodine, should give the most comprehensive picture of changes in iodine balance and distribution in the body.

The use of such stable and radioiodine techniques was

attempted in the present study of the balance of body, environmental and dietary iodine in rainbow trout, (Salmo gairdnerii).

MAINTENANCE OF EXPERIMENTAL ANIMALS

Fish used throughout this study were rainbow trout, obtained from the Alberta Department of Lands and Forests fish hatchery. The fish were healthy when received and prospered on the dry pellet diet provided (Clark's New Age Fish Feed, J. R. Clark Co., Salt Lake City, Utah). Analysis of two separate batches of the diet showed them to contain 2.88 and 2.66 µg of iodide/g dry weight. The fish were acclimated and held at experimental temperature (10 or 15° C) in 720 litre tanks. The photoperiod was ten hours.

Routine water analyses of the dechlorinated water supply, provided by the Alberta Provincial Analyst, had the following average concentrations for 1962-63 (as p. p. m.): T. D. S. - 165. 5, ignition loss - 43, hardness - 85. 0, sulphates - 50. 0, chlorides - 3. 5, alkalinity (as calcium and magnesium bicarbonates) - 46. 0. The concentration of nitrates, nitrites and fluorides was nil and iron was present only in trace amounts. Iodine analysis carried out in our laboratory in the late spring of 1963 showed a total iodine concentration of 0. 087 µg/l in the dechlorinated water supply.

METHODS AND MATERIALS

A. Administration of pretreatments:

The major regimens and water conditions used will be described here in detail and referred to later in the results. Other treatments or pretreatments will be described in the results.

a. Controls (C). Control animals were fed the standard dry pellet diet supplied to fish retained in the stock-holding tanks. Water provided came from the regular laboratory dechlorinated supply.

b. Iodine-enriched water (IR). Dechlorinated tap water was enriched by the addition of potassium iodide, to bring the iodide concentration to 30 µg. of iodide/l above control levels. Exposure to water of this iodine content was limited to 36 hours plus the period of time necessary to complete the radioiodine analyses. Feeding was scheduled as for the controls.

c. Iodine-poor water (IP). The supply of dechlorinated water was passed through a bed of anionic exchange resin (Amberlite IRA-400, Rohm & Hass Co., Philadelphia), which raised the pH of the water to 11.0. The pH was then adjusted to 7.5 with a mixture of H_2SO_4:HCl = 3:1. Fish were maintained in these conditions for 36 hours*, plus the length of the radioiodine administration studies.

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* Tap water passed through an anionic exchange resin, as used in this experiment, is fatal to rainbow trout in less than 12 hours. However, near neutralization of the strongly basic water with mineral acids (sulphuric and hydrochloric) allowed survival for periods in excess of two weeks. Deionized water contained only 0.0013 micrograms of iodide/litre, while ordinary dechlorinated water contained 0.087 micrograms of iodide/litre.

d. Starvation (St). Starvation was limited to 48 hours, to reduce mortality, in addition to the length of time required to complete experimental observations. Fish that were starved, as well as pretreated with iodine-enriched water (IR-St), were placed in the iodine-enriched water 12 hours after starvation began. Thirty-six hours later, radioiodine experimental treatment was commenced.

B. Administration of radioiodine:

Throughout the study an effort was made to avoid experimental procedures that would entail frequent or rough handling of the trout, or the subjection of trout to other stressing conditions. When repeated handling was unavoidable, as, for instance, during serial observations or repeated injections of individual fish, anaesthetic (MS-222, Sandoz Co., Basle) was used.

Radioactive iodine (I^{131}) was administered through natural routes of iodine passage (by addition to the water or by administration with the food) in preference to administration by injection.

Dosages of radioiodine were kept small to minimize any radiation effects, especially in the small fish. An I^{131} concentration of 15 μc I^{131}/l of water was used in most experiments. I^{131} concentrations of 15 and 50 μc /l were used in the study of I^{131} gill uptake, using radioautographic methods. Exposure was for one hour, or two to ten minutes for the higher concentration.

In the study involving dietary I^{131}, the dry pellet fish food was administered as a thick paste with radioiodine added at a concentration sufficient to give each fish an initial whole body activity of 8,000-10,000 counts per minute in a well crystal scintillation counter.

Administration of radioiodine by injection was used only with large fish (50 ± 3 gms.) The initial dose was five microcuries per fish and radioactivity was restored to initial levels by daily injections of smaller amounts of I^{131}. All injections were made forward into the coelom, by passing the needle through the heavy musculature of the caudal peduncle.

C. Sampling and counting procedures:

Serial in vivo counting of small radioactive fish was done in a well crystal scintillation counter. Anaesthetized fish were individually placed, head down, in a counting tube, which contained sufficient water to cover the end of the tail. Fish were generally only counted for one minute. However, if a longer count was required, the fish was counted for one minute intervals, the water in the tube being changed between countings. It was rarely necessary to count for longer than two minutes.

Radioiodine counting of fish and thyroids to be analysed for stable iodine were carried out in moist, empty counting tubes; then the tissues were removed, wrapped in aluminum foil and frozen. If only radioiodine analysis was necessary, tissues were dissolved in equal volumes of concentrated nitric acid before counting in a well crystal scintillation counter.

For the estimation of relative distributions of I^{127}, I^{131} and chloride, all tissue samples were counted in tared, parafilm-covered stainless-steel planchets, positioned on a lead plate 11.5 cm. below a Nuclear-Chicago end-probe scintillation counter. The tissues analyzed were: whole blood, plasma, skin, epaxial trunk muscle, gill, thyroid, liver, spleen, kidney, brain and samples of the homogenized remainders

of the carcasses. After weighing, all similar organs except muscle were pooled, homogenized,and the homogenates were analyzed for total stable iodine and total chloride.

D. Analytical methods:

a. Histology and radioautography. Fish were removed from the I^{131} solution, rinsed briefly in fresh water and then killed. Sections of gillbars with their attached gill lamellae, pseudobranch, and the more vascular areas of the inner side of the operculum were immediately removed and treated by either one of two methods: (1) Standard fixation - tissues were fixed in very hot Heidenhain's Susa fixative, then prepared by conventional techniques for paraffin sectioning. Since any fixative may leach unbound iodide from tissue, a strong mercurial fixative was chosen, in the hope of precipitating soluble iodide in situ, thus preventing the appearance of artifacts and leaching sometimes associated with the use of mercurial fixatives in the preparation of radioautographs (Comar, 1955). However, no leaching or artifacts were encountered. (2) Freeze-drying - Thin pieces of tissue were rapidly frozen between flat pieces of dry ice. The tissues were transferred to a cold stainless-steel basket on a chilled steel plate, equipped with a greased rubber gasket. A small glass funnel was attached to the plate gasket by vacuum produced by a high-vacuum pump. The whole funnel assembly was submerged in a dry ice-alcohol bath for 24-36 hours. After warming to room temperature, dry air was admitted to the assembly and the tissues were placed in molten paraffin for embedding and sectioning.

Mounted sections prepared by both methods were coated

on one side by dipping in Ilford G-5 Nuclear Emulsion for the preparation of radioautographs. When the emulsion was dried, slides were stored in a refrigerator at 5° C., in a light-tight box, containing a packet of drying agent. A thin celloidin film between the sections and emulsion was used, but produced no visible improvement in the radioautographs. Fogging and excessive background were minimal. After development, the tissues were stained through the emulsion with Ehrlich's hematoxylin and eosin for microscopic examination.

b. Stable iodine analysis. The method of iodine analysis used in this laboratory is an adaptation of Acland's (1957) method. The method utilizes the catalytic properties of iodide to promote the arsenite reduction of orange ceric ions to the colourless cerous form.

For analysis of total iodine content, the samples used (in most cases thyroids and whole bodies of small fish) were dissolved in one ml. of 4N-NaOH and one ml. of absolute ethanol, prior to drying and incineration. Total plasma iodine was determined on samples of serum which were dried and incinerated with one ml. of 4N-Na_2CO_3.

Protein-bound iodine (PBI) was determined from zinc hydroxide-precipitated plasma proteins, washed several times with double-distilled water, prior to digestion and incineration with one ml. of 4N-Na_2CO_3. The supernatant washes from the PBI were retained and used in the determination of inorganic (I I) iodine.

Butanol extractable (BEI),or thyroxine-like iodine, was determined as follows:

Plasma was extracted with several washes of n-butanol (acidified with a small amount of 2N-HCl) to remove iodide and organic iodine compounds. Extraction of the butanol with 4N-NaOH in 5% Na_2CO_3 removed all iodine compounds except thyroxine and triiodothyronine. The butanol extract was dried and incinerated with one ml. of 4N-Na_2CO_3.

In all cases, reagent blanks identical to the sample reagents were used to correct for any iodine present in the reagents, which were analytical reagent grade dissolved in glass distilled water.

After incineration, an acidified water extract of the sample ash, containing arsenious acid was incubated at 37° C.; ceric ions were added and the reaction allowed to proceed for 12.5 minutes. At this time, percent transmittance at a wavelength of 415 mu. was measured with a Bausch and Lomb Spectronic 20 colourimeter. Interpolation of this reading into a calibration curve of known iodine concentrations, prepared simultaneously with the unknown sample, allowed calculation of iodine content of the sample after a correction was made for the reagent blank.

c. Chloride analysis. The analysis of total chloride was performed on wet tissue, using an alkaline digestion method, described by Cotlove (1963). Coulometric-amperometric titration with a Buchler-Cotlove Chloridometer (Buchler Instruments, Fort Lee, N.J.) allowed accurate analysis of the chloride present. Concentrations were expressed as tissue/plasma ratios.

d. Chromatography. Pieces of muscle-free dorsal skin, from above the lateral line, were incubated at environmental temperature (10° C.) for four hours, in Young's fresh water teleost saline (Hale, 1958), which

contained 250 μc. of I^{131}/ l. Both oxygen and air were bubbled through the saline, the rate of gas flow being just sufficient to keep the skin slowly circulating about to prevent folding and sticking.

After incubation, the skin was blotted to remove excess moisture, then homogenized in a glass-teflon homogenizer. After extraction by three washes of n-butanol acidified with 2N-HCl, samples of extract were analyzed by descending one-dimensional paper chromatography (Whatman No. 1 chromatography paper; n-butanol:dioxane:ammonia = 4:1:4)(Pitt-Rivers and Tata, 1959; Block et al., 1958; Bois and Larsson, 1958). All extract samples, both with and without thyroid hormone carriers, were accompanied by control strips. The filter paper strips were scanned for radioactivity with a thin mica window gas-flow geiger counter, coupled with a continuous scanner and recorder.

RESULTS

Two experiments (A and B) were designed with a view to establishing the relative contribution of environmental and dietary iodine to the body iodine pool.

A. Iodine uptake from the water environment:

Four groups of rainbow trout (0.2-0.7 g body weight), acclimated to 15° C., were pretreated as outlined below, prior to the addition of a tracer quantity of I^{131} to the water.

Pretreatments were as follows:

(1) Controls (C)

(2) Iodide-enriched water (IR)

(3) Starved (St)

(4) Starved in iodide-enriched water (IR-St)

Serial samples of total body I^{131} uptake and thyroidal I^{131} accumulation were measured. Bodies and thyroids were analyzed for stable iodine content.

Thyroid uptake of radioiodide was markedly lower in the two groups of fish held in iodide-enriched water than in the two tap-water groups. Iodine content of the water was the major factor that distinguished the four groups. The effect of diet was less pronounced and resulted in only minor differences between the groups held in each of the different iodine concentrations (Fig. 1).

The stable iodine contents of the bodies and thyroids, as well as the calculated concentrations of the whole bodies (body+thyroid) are given in Table I. Mean iodine concentrations are expressed as μg

of iodine/100 g of tissue.

The whole body concentrations of iodine of the two groups held in iodine-enriched water are significantly different (IR-St>IR; $t = 2.87 > t_{.05} = 2.11$.) In the groups held in iodine-poor water, the mean whole body concentrations are significantly different at the 10% level of probability, but not at the 5% level (C>St; $t_{0.1} = 1.74 < t = 1.97 < t_{.05} = 2.11$). (See Table I). Both groups held in iodine-enriched water are significantly greater than the unenriched water groups (IR> C; $t = 2.60 > t_{.01} = 2.56$ and IR-St>St; $t = 2.99 > t_{.01} = 2.54$).

Changes in whole body stable iodine concentrations are shown in Fig. 4. Analysis of covariance shows that no significant difference exists between the adjusted means of the regressions of fed and starved fish in dechlorinated water ($F=0.30 < F_{0.1} = 2.84$). There is also no significant difference in the regressions of the lines plotted for fed and starved fish in iodine-enriched water ($F=0.76 < F_{0.1} = 2.84$). Regressions for starved fish are not significantly different from zero slope (St-F=0.68$<F_{0.1}$=3.03; IR-St-F=0.46$<F_{0.1}$=2.96). Despite lack of statistical significance in the changes of iodine concentration with time, these data show that trends are established with time under different conditions of water and diet.

Consequently, it appears that the major proportion of iodine utilized by rainbow trout is derived directly from the water, despite the small concentration of this element in fresh water. Although food contains relatively large amounts of iodine, it is a less important source of iodine. Another point of considerable interest is the difference between the reactions of thyroidal and extrathyroidal tissues to conditions of iodine enrichment. The effect of increased iodine availability is much greater stability in the concentrations of thyroid iodine than is found in the concentrations of body iodine(see Table I). Iodine enrichment of the water increase the thyroidal concentration of I^{127} in the fed fish by only 12%, but body concentratio increases almost 300% , Similarly, in the starved groups, thyroid concentration increases 109%, while the body concentration increases by over 530%.

TABLE I

Stable iodine (I^{127}) concentrations of starved and fed fish in waters of different iodine concentrations.

	Iodine-Poor (Tap) Water	Iodine-rich (30 µg/l.) Water
Fed	Body 7.54±0.78*(8)** Thyroid 149.51±38.2 (8) Whole Body 13.16±0.89 (20)	Body 30.03±2.65 (15) Thyroid 167.92±25.8 (15) Whole Body 34.89±2.46 (15)
Starved	Body 7.20±0.81 (18) Thyroid 100.43±7.85 (18) Whole Body 10.99±0.66 (18)	Body 45.41±4.59 (23) Thyroid 209.62±33.82 (23) Whole Body 50.03±4.62 (23)

* Mean± S.E.
** Sample size

This difference in behaviour strongly suggests that, within limits, the thyroid is capable of regulating its total iodine content. On the other hand, extrathyroidal iodine seems to vary passively, in accordance with the iodine availability.

B. Effects of environmental iodide on dietary uptake of I^{131}:

Groups of fingerling rainbow trout (1-2 g body weight) were pretreated in the following ways (see Methods and Materials - Pretreatment and Radioiodide administration sections):

(1) Controls (C)

(2) Iodide-enriched water (IR)

(3) Iodide-poor water (IP)

Figure 1. Percent of whole body I^{131} dose in thyroid after accumulation from the water. Identifying abbreviations are the same as used in the text. Each point represents the average of two to five fish. Zero hours is the completion of 48 hours starvation and/or 36 hours in iodine-enriched water.

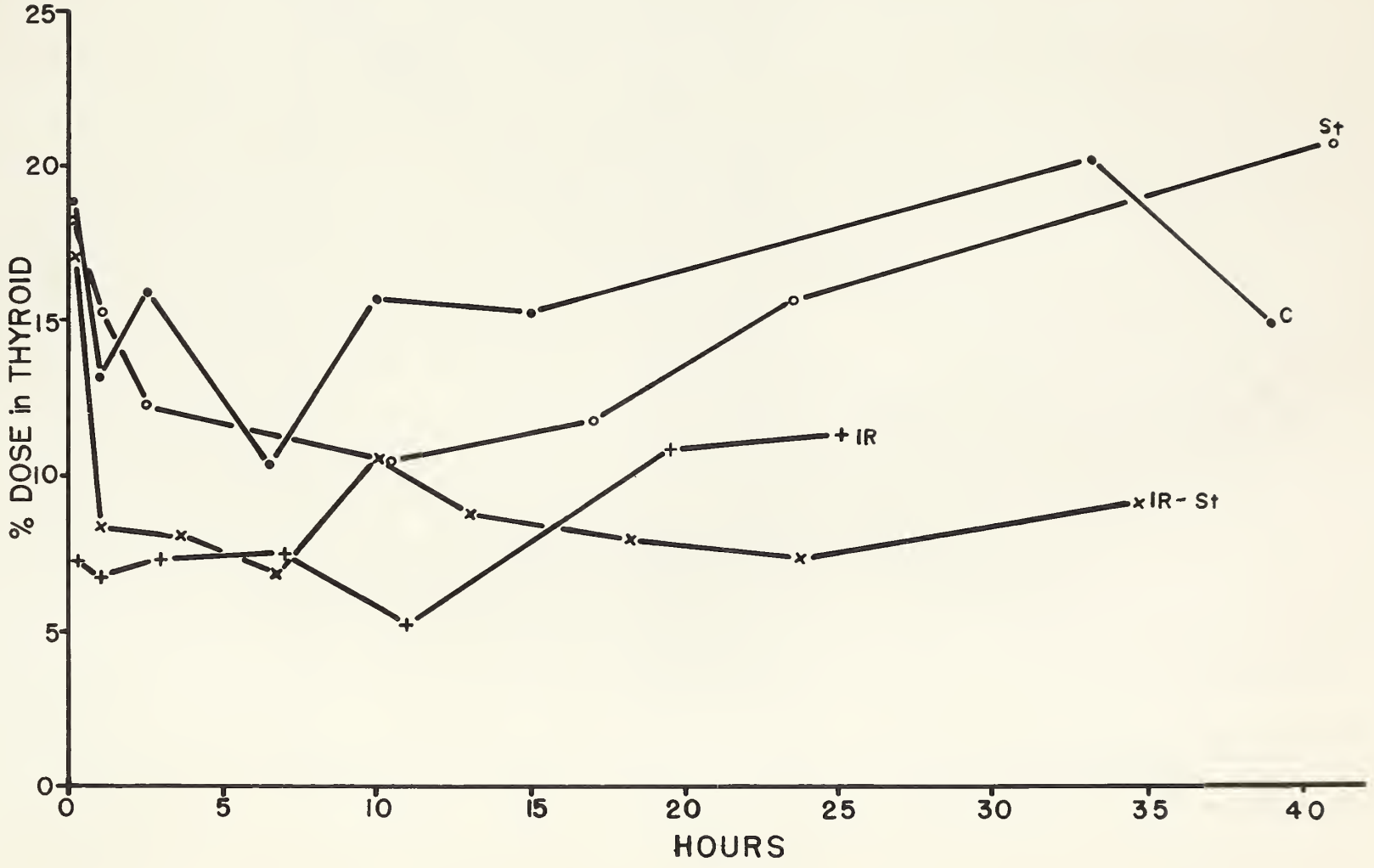

25
20
15
10
5
0
% DOSE in THYROID
0
5
10
15
20
25
30
35
40
HOURS
St
C
+ IR
x IR - St

Figure 2. Rate of disappearance of a dietary dose of I^{131}. Closed circles, solid line - controls; open circles, dotted line - iodine-poor water; split circles, dashed line - iodine-enriched water. Vertical lines - plus and minus one standard error of the mean; each point is average of six fish.

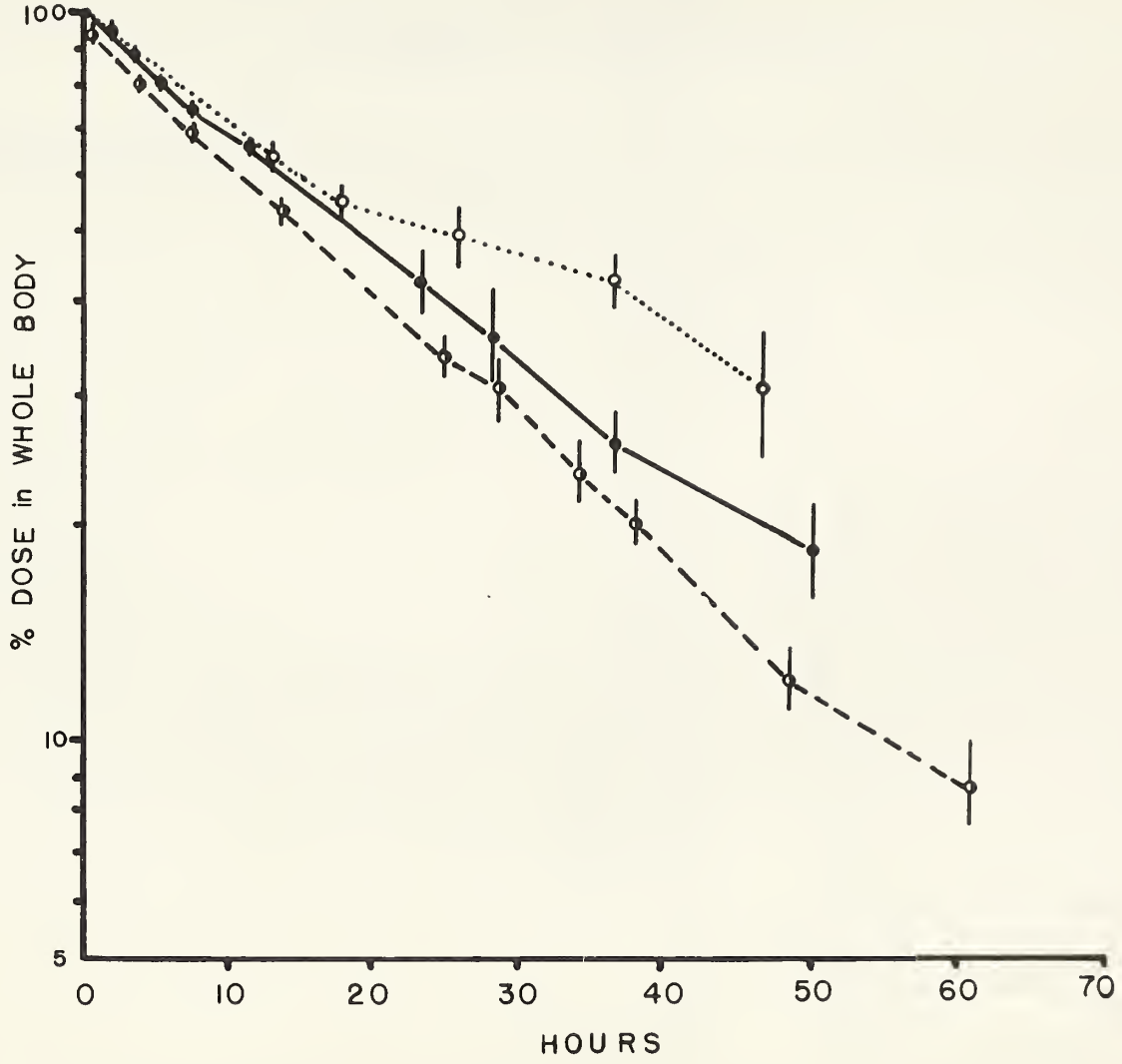

100
10
5
% DOSE in WHOLE BODY
0
10
20
30
40
50
60
70
HOURS

Figure 3. Expansion of the radioiodide space as percent body weight (closed circles) and blood: environment concentration ratio (B:E) (open circles). Each point is the average of two or three fish.

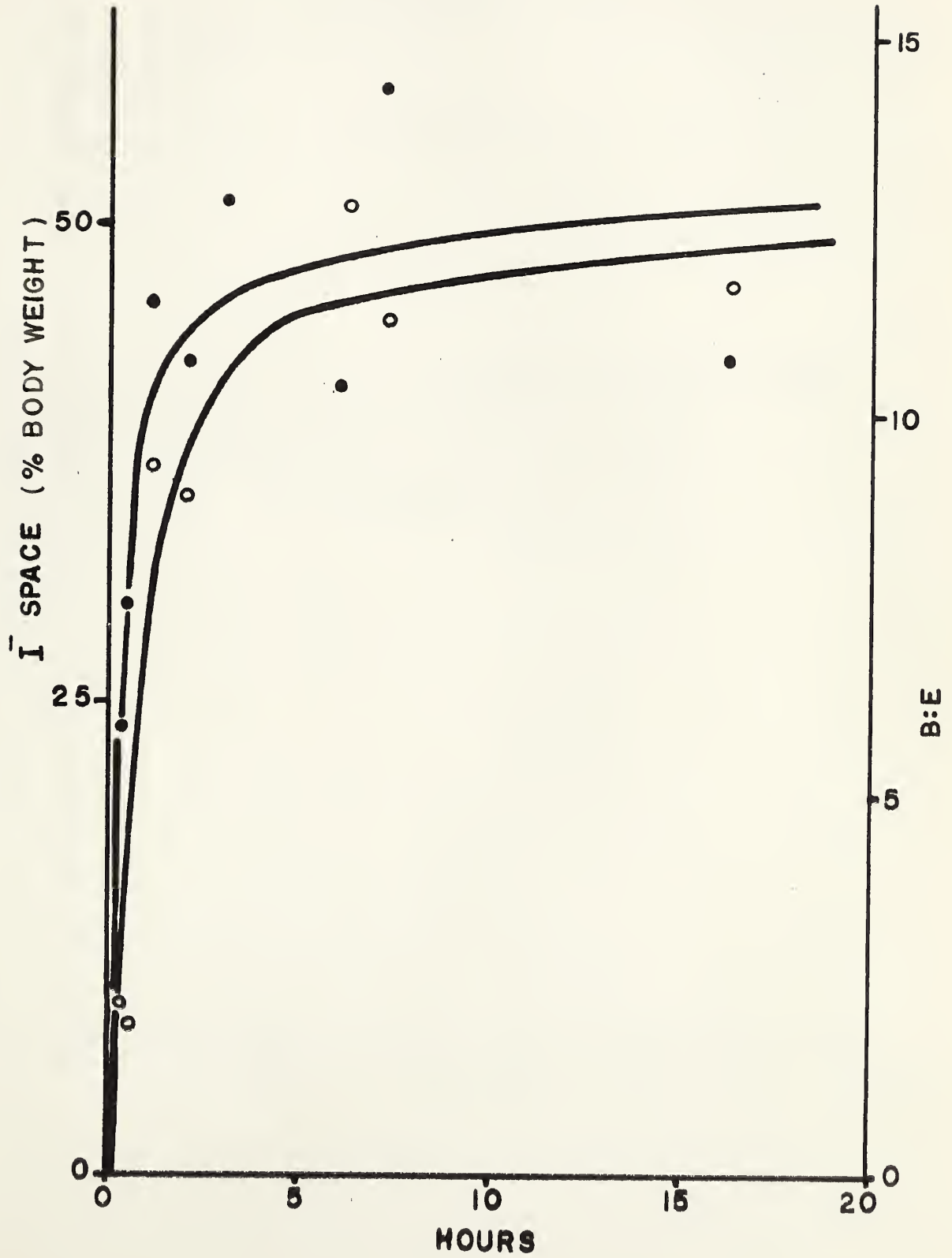
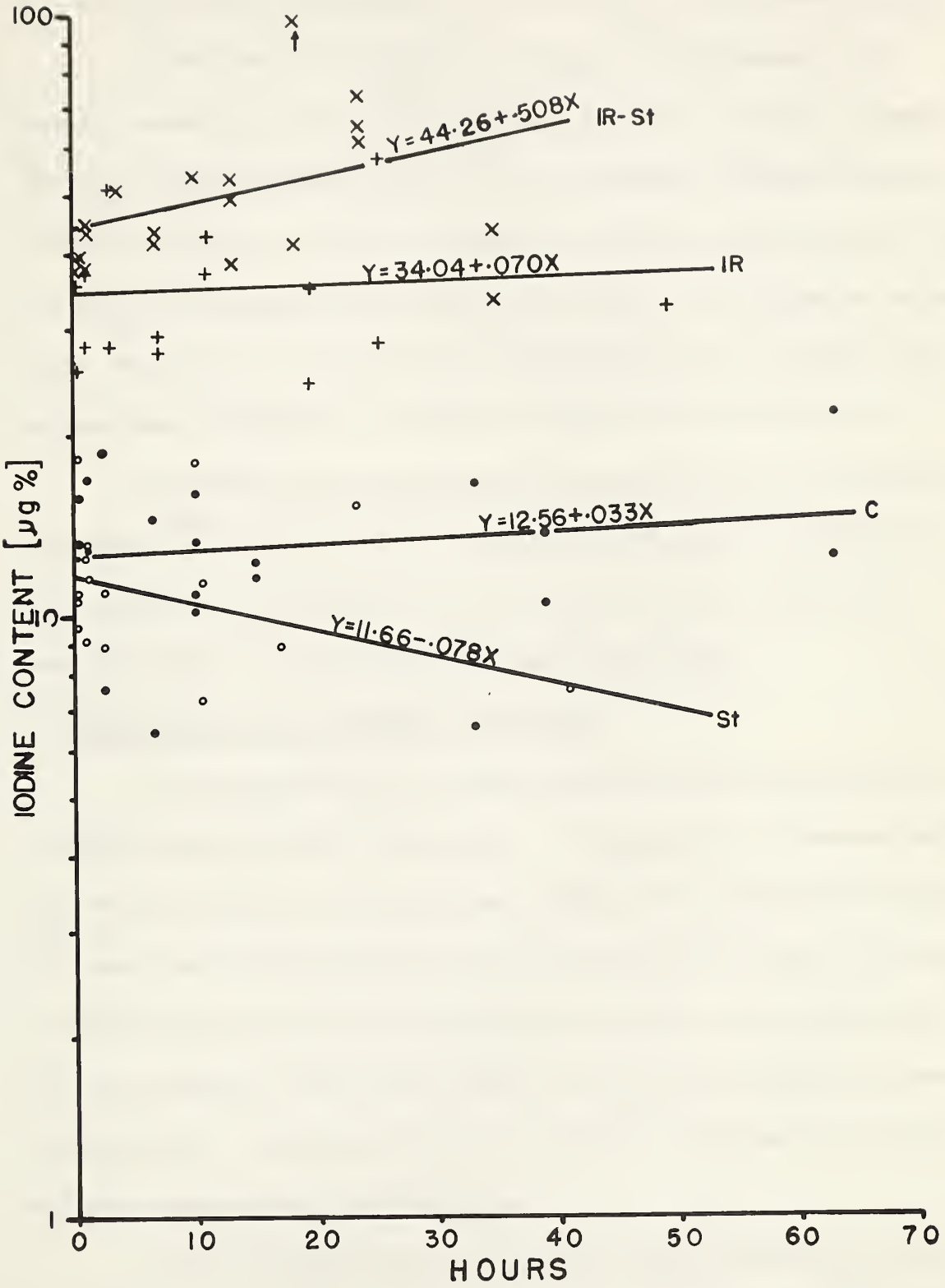

$\bar{I}$ SPACE (% BODY WEIGHT)
50
25
0
0
5
10
15
20
HOURS
15
10
5
0
B:E

Figure 4: Whole body iodine content (I^{127}). Zero hours represents the completion of 48 hours starvation and/or 36 hours in iodine-enriched water. Identifying abbreviations are the same as used in the text. C - closed circles; St - open circles; IR - crosses; IR-St - exes (as in Fig. 1).


100
10
1
IODINE CONTENT [µg %]
Y=44·26+·508X
IR-St
Y=34·04+·070X
IR
Y=12·56+·033X
C
Y=11·66−·078X
St
0
10
20
30
40
50
60
70
HOURS

After completion of pretreatment, the fish were fed a dose of I^{131} and the rate of disappearance from the body was followed in vivo.

In each of the three different groups, disappearance of the dietary radioiodine occurred in nearly a logarithmic manner, although each group lost their dose of I^{131} at a rate distinctly different from the other two groups. Forty-five to fifty hours after the dose was fed, those fish held in iodine-poor water had excreted only about 70% of the initial dose. By this time, control fish had excreted slightly over 80% of the original dose and the fish in iodine-rich water almost 90% (Fig. 2).

The effect of environmental iodine availability on the retention of dietary I^{131} is unequivocal. When iodine is abundant, excretion of dietary iodine is increased; in waters poor in iodine, retention of available iodine is promoted and excretion minimized.

C. Penetration of environmental radioiodide:

Little information is available concerning the characteristics of iodide uptake from the environment. Consequently, to become familiar with the penetration of environmental iodide into the bodies of normal fish, I^{131} was used to trace the expansion of the radioiodide space of the body, the rate of accumulation of radioiodine in the body and thyroid and the I^{131} concentration in the blood relative to environmental and thyroidal concentrations. The average rate of water I^{131} clearance by the blood was also investigated from these data.

The radioiodide space (as percent of body volume) can be calculated as: I^{131} space = $\frac{I^{131} \text{ concentration in body (without thyroid)}}{I^{131} \text{ concentration in blood}} \times 100\%$

Fingerling rainbow trout (1-2 g body weight) were used, following

the procedure outlined for radioiodine analysis.

During the first hour of immersion in the radioiodide solution, expansion of the radioiodide space was rapid, reaching a value greater than 40% of the body weight. After this time, the rate of expansion slowed. The blood:environment ratio followed a similar pattern, reaching a value of 8-10 in the first hour. Further increase was slow (see Fig. 3).

The radioiodine accumulation in blood, body and thyroid (as counts/minute (CPM)/g) followed a very similar pattern, as shown especially by the thyroid/blood ratio, which averaged 1.25 throughout the entire duration of the study.

The average rate of water I^{131} clearance by the whole body is 31.8 ml /hr /g of body weight over the first five minutes. This rate, measured at a time when there is little I^{131} excretion back into the water, is an approximation of the steady state rate of entry of environmental iodine into the body.

D. Uptake of I^{131} by gill tissue:

The fact that the gills of fish can transport chloride and other monovalent ions from the water around them is well substantiated (Krogh, 1937; Black, 1957). It is also known that all halides in the vertebrate body are similar in distribution and behaviour (Baumann and Metzger, 1949; Danowski, 1962; Wallace and Brodie, 1937; Yagi et al., 1953). To test the hypothesis that chloride cells of the gills of fish may actively transport iodide from the environment, in the same way that they transport chloride, an autoradiographic study of histological preparations from the gills, pseudobranchs and inner operculum of rainbow trout was carried out. The methods have been outlined in a previous section.

No cellular localization of radioiodine was detectable in the gill lamellae, although a large amount of radioactivity was present in the cartilaginous gill bars. No accumulation of I^{131} was discernable in the cells of the pseudobranchs, or cells of the inner side of the operculum.

It may be concluded, then, that this autoradiographic method failed to demonstrate any active transport of iodide by the chloride cells of the gills of rainbow trout.

E. Ingestion of water:

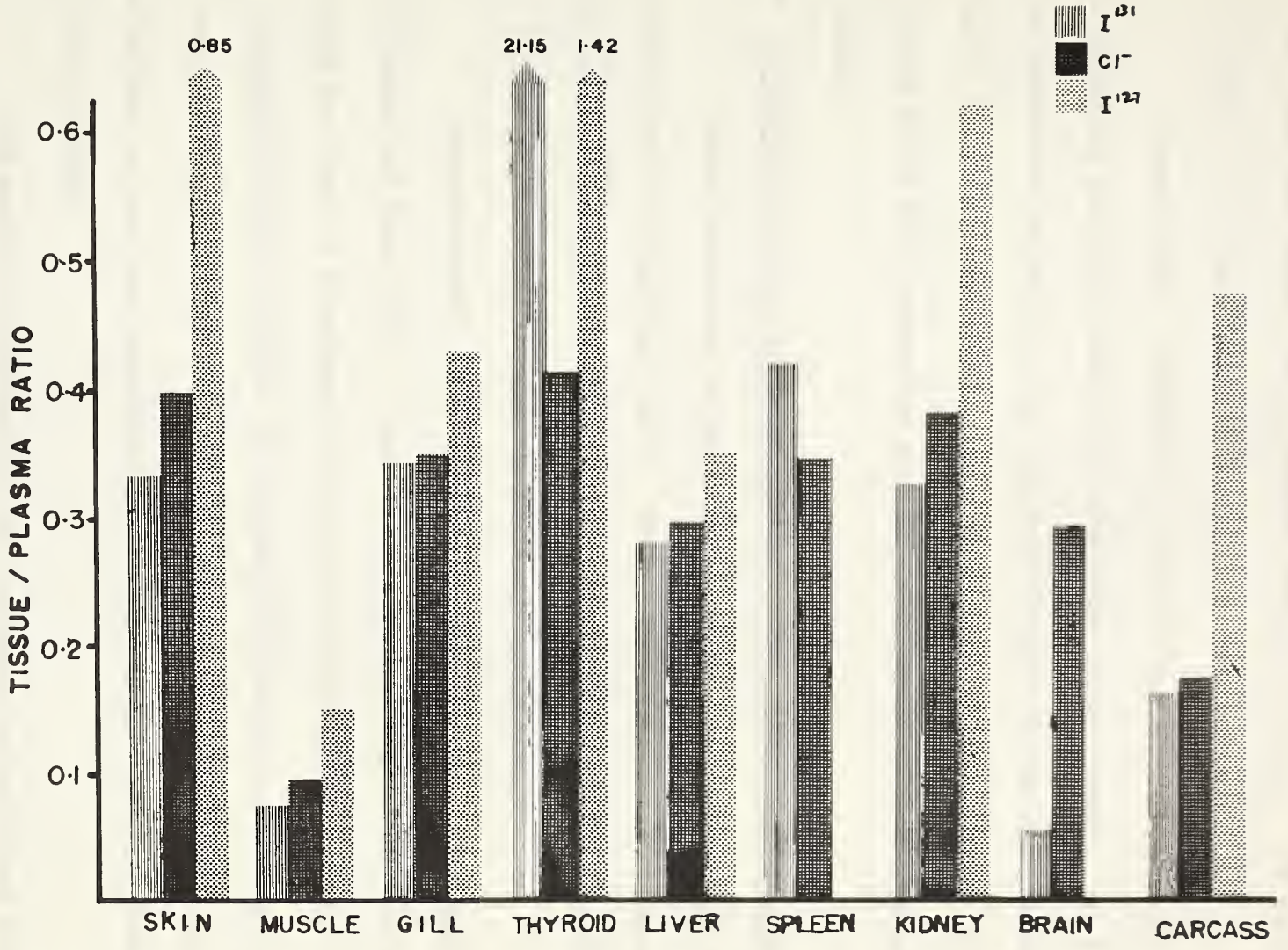
The work outlined in the preceding sections has shown that environmental iodine is an important source of iodine. However, it failed to show how this iodine entered the body. Consequently, an estimate of the amount of water ingested by rainbow trout was desirable.

Two groups of fish (four fish each) were immersed, without feeding, in water that was darkly colored with vital red or Evan's blue (T-1824) dyes. At intervals fish were removed, killed, and the alimentary canal was dissected out.

The contents of the stomach and intestine were expressed onto a piece of absorbent white paper, and the moisture was allowed to seep into the paper. The intestine and stomach were split open and applied to the paper until all moisture was absorbed. Then the gut walls, their contents, and the paper stains made by each were examined for traces of coloration by the dyes.

Although the fish were deeply stained about the mouth and opercula, no dye coloration was found in the gut or its contents during periods up to 96 hours.

Figure 5. Distribution of I^{127}, I^{131} and chloride in tissues expressed as tissue/plasma concentration ratios. Iodine^{131} is the average of six samples; others are values of pooled tissue from six fish.


I^{131}
Cl^-
I^{127}
0·85
21·15
1·42
TISSUE / PLASMA RATIO
0·6
0·5
0·4
0·3
0·2
0·1
SKIN
MUSCLE
GILL
THYROID
LIVER
SPLEEN
KIDNEY
BRAIN
CARCASS

In only one specimen was any dye found. A very slight trace of Evan's blue was found in the gelatinous stomach contents. No coloration was found in the stomach walls, the gut, or its contents.

These results, therefore, agree with earlier work (Krogh, 1939) in indicating that fresh water fish drink little, if any, water. As a result, the acquisition of iodine by the ingestion of water may be considered unimportant to the total iodine turnover in rainbow trout. Consequently, iodine present in the water enters the body by direct penetration of permeable tissues.

F. Distribution of total iodine in relation to total chloride content of tissues:

Iodide behaves as a typical extracellular ion, when administered in large doses to mammals (Sacks, 1953). However, at either pharmacological or physiological concentrations, little is known of the distribution of iodide, or the other halides, in the lower vertebrates.

Extrathyroidal iodine depots have been reported in Fundulus (Gorbman and Berg, 1955). Thus, if any extrathyroidal concentrations of iodine are found, a knowledge of chloride distribution will reveal if this is restricted solely to iodine, or whether other halides are present in increased concentrations. The same case is true of decreased concentrations of halides, as has been reported for non-chloride halogens in the central nervous system.

See methods and materials for procedure and analytical methods.

The relative distribution of iodide-131, iodide-127, and chloride are shown graphically in Fig. 5 as tissue:plasma (T/S) ratios. With the

exception of brain, thyroid and carcass, the distribution in the tissues examined is similar for all three ions, although the muscle concentrations are much lower than other tissues. In thyroid, skin, kidney and gut, iodine ratios are much higher than the corresponding values for chloride. In brain tissue, chloride levels are comparable to other tissues, but iodine concentrations are very low. The T/S ratio of red blood cells averaged only 0.08.

Sacks (1953) noted that iodide in high concentration acted as a typical extracellular ion. These data show that, with the exception of brain, thyroid, and part of the carcass (probably the gut), iodide acts as an extracellular ion at physiological concentrations, as well.

In order to give some estimation of the different blood iodine components, an additional plasma sample from trout of comparable body size was analyzed for different iodine fractions. The different concentrations (as μg. %) were: Total - 28.36; protein-bound iodine (PBI) - 2.53; thyroxine-like or butanol extractable iodine (BEI) - 2.45; inorganic iodine (II) - 25.83.

Original total plasma iodine, as used in Fig. 5, was 46.9 μg. %.

G. I^{131} metabolism by isolated fish skin in vitro:

An analysis of the movements of iodide across the skin of fishes is essential to a complete outline of iodine sources available to fish. Due to technical difficulties and time limitations, an estimate of iodine entry into the body via the skin was unsuccessful. However, some preliminary work on the iodine metabolism of the skin in vitro was completed and is reported here.

For chromatographic procedures, the solvent system employed (butanol-dioxane-ammonia) gave excellent separation of the thyroxine analogues. The Rf values for thyroxine, 3:5:3 triiodo-L-thyronine, 3:5 diiodo-L-tyrosine and 3 iodo-L-tyrosine, used as standards and carriers, were 0.55, 0.70, 0.15, and 0.21, respectively.

The thyroxine radioanalogues were somewhat variable in their relative concentrations in the skins of different fish. However, all four analogues used as standards and carriers were found to be present in the extracts of the incubated skins.

In addition to these four identified analogues, three other peaks of radioactivity were present as contaminants from the radioiodine solution. Only one of these disappeared from the extracted tissue, the others remaining relatively stable. In addition, one unidentified compound was present in the extract, with Rf = 0.80 - 0.84.

It is concluded that extrathyroidal formation of thyroid hormones occurs in fish, perhaps mainly in areas of melanin pigmentation where tyrosine is abundant. Unfortunately, the amount of iodine entering the body via the skin is still unknown.

DISCUSSION

A. Tissue I^{131} content; water iodine clearance:

The initial sharp increase in the tissue radioiodine concentrations demonstrates the rapid accumulation of I^{131} in these tissues (blood, body and thyroid), until the specific activities of water and the different tissues have come into equilibrium. As equilibrium of body and environment concentrations is approached, the rate of accumulation decreases; at true equilibrium, net flux is nil. The rate of accumulation of iodide by the different tissues is very similar in form to the rate of expansion of the radioiodide space, or changes in the blood:environment ratio, as shown in Fig. 3.

The ambient water is cleared of radioiodine at a rate that decreases asymptotically as equilibrium is approached. Eventually, environmental I^{131} influx becomes equal to total I^{131} excreted. The initial rate of radioiodine clearance from the water (31.8 ml of water/hr/g of body weight during the first five minutes of immersion) is an approximation of the steady state rate of iodine removal from the water, since little excretion has occurred. Iodine is excreted from the body at an almost identical rate. In water of average iodine content (about 0.1 μg of iodine/l of water), iodine enters and leaves the body at a rate of about 0.077 μg. I^-/day/g of body weight.

B. Expansion of I^{131} space; B:E ratio:

The radioiodide space is an expression of the apparent volume of distribution (or dilution) of radioiodide in the body. It is not a separate physical compartment, but is distributed largely extracellularly, as are

sodium and chloride (Sacks, 1953).

The apparent volume of the body not penetrated by iodide (Fig.3) corresponds closely to the mass of skeletal muscle present in the bodies of teleost fish (50-60% of body weight). In contrast to this limited iodide space, and the slow penetration of iodide into skeletal muscle, the radioiodide space of marine starry flounder reaches 100% in approximately 32 hours (Hickman, 1959), indicating rapid cellular penetration of iodide.

There are two major sources of error present in an estimation of radioiodide space. Error can be introduced into the space estimate if a substantial amount of organified radioiodine is returned to the circulation, resulting in high body radioactivity that is not due to inorganic radioiodine. The removal of any freely diffusable iodide present in the tissue removed with the thyroid will also contribute a small negative error to the radioiodide space. Both of these sources of error are probably insignificant here, since the duration of the experiment was short and all thyroids were carefully trimmed of extrathyroidal tissue.

There is a sharp concentration gradient of iodide between the blood and environment, the blood:environment ratio (B:E) reaching a maximum value of about 12 (Fig. 3). However, throughout the experiment, the total concentration of radioiodine in the thyroid was only 25% above blood concentration (thyroid/blood I^{131} concentration ratio = 1.25). In normal mammals, the I^- ratio is approximately 25 (Slingerland, 1955; Halmi et al., 1953).

The rate and degree of iodide trapping by the thyroid are directly dependent on the concentration of iodide in the serum, which in turn is

dependent on environmental iodine availability. Consequently, this work supports Hickman's (1962) observation that iodide concentration mechanisms of great importance to fish reside in the organs of ionic exchange between body and environment, as well as in the thyroid gland.

In salmonid fishes, inorganic iodide in the blood is extensively bound to plasma proteins, with little iodide found as free ions, or in red blood cells (Leloup and Fontaine, 1960). Extensive binding of inorganic iodide to plasma proteins, a phenomenon apparently absent in homoiotherms (Riggs, 1952), may be instrumental in the acquisition of iodide from the environment, especially since active transport of iodide from the environment does not seem to occur. This binding would serve a dual purpose, by preventing substantial loss of iodide across the gills or excretion by the kidneys (Leloup and Fontaine, 1960), and by making the effectively diffusable concentration of unbound iodide in the blood so low that iodide could diffuse into the vascular bed by a simple concentration gradient. If such a mechanism exists, there is then introduced the problem of how the release of iodide from plasma protein binding for utilization by the thyroid could occur. The apparent absence of mono- and di-iodotyrosines from the blood (see Section C) is indicative of an effective deiodinating system in the thyroid. It is possible that a similar system for the deiodination of plasma proteins of the blood exists in the thyroid of rainbow trout.

In summary, radioiodine is slow to penetrate the entire mass of the body of rainbow trout. Although thyroid and blood concentrations of I^{131} equilibrate rapidly, the value of the thyroid/blood ratio is much lower than in mammals. In addition, iodine is accumulated by the blood at a concentration

well above that of the environment.

C. Distribution of total iodine in relation to total chloride content of tissues:

In most tissues of the body, the distributions of chloride and iodide are in equilibrium with serum concentrations of these ions. However, certain exceptions are found (Fig. 5).

The concentration of iodide in the fish thyroid gland, under conditions of iodide equilibrium (Fig. 5, I^{127}; also see Section B) is close to the concentration of iodide in serum, the tissue:serum ratio reaching a value of 1.2-1.5. The elevated tissue/plasma ratio of thyroidal I^{131} is probably indicative of a lack of complete equilibrium of I^{131} as a consequence of the periodic administrations. Very little radiohormone is present in the circulation or extrathyroidal tissues until at least six days after the initial administration (Eales, 1963), resulting here in a fallaciously high tissue/serum ratio value for thyroid.

The very low value of the total I^{127} thyroid/serum ratio (1.42) suggests that the major function of the trout thyroid is organification of iodide. Iodine values of the thyroid tissue were near the limit of sensitivity of the I^{127} analytical method, due to large sample size, but there is no reason to expect an error of more than ten percent. Consequently, it seems that iodide may diffuse passively into the gland. The only method of iodine concentration available would then be protein binding (probably to thyroglobulin) inside the thyroid follicles.

The mammalian thyroid gland is known to accumulate other halogens (F, Br, Cl) as well as the heavy metals Re, Mn, and Tc (Baumann and Metzger, 1949; Baumann et al., 1949; 1953) although no

organification occurs (Pitt-Rivers and Tata, 1959; Gross, 1962). The thyroidal accumulation of elements of the seventh periodic groups (7a and 7b) gives these elements varying degrees of goitrogenicity when their concentration is sufficient to produce a mass action (washing-out) effect (Baumann and Metzger, 1949; Isler et al., 1958). The lack of conspicuous concentration of chloride by the thyroid of rainbow trout is probably due to the small, diffuse nature of the gland. Enough extrathyroidal chloride was present in the thyroid samples to dilute any thyroid tissue chloride to low levels.

High concentrations of stable iodide (I^{127}) are present in skin, kidney and carcass. The lack of similar concentrations of I^{131} in these tissues probably results from a lack of I^{131} equilibrium. Part of the high stable iodine concentration of the carcass results from food in the gut. However, additional iodine is probably present in the biliary secretions (Intoccio and Van Middlesworth, 1958; Van Middlesworth, 1956; Sasaki and Nakajima, 1962), as well as some iodide that is transported into the gut as a consequence of chloride pumping (Pastan, 1957; Lipner and Hazen, 1962). Radioiodine concentration in the walls of the frog tadpole gut has been verified autoradiographically (Dent and Hunt, 1952).

No stable iodine determinations were possible for brain, but a reasonable estimate of the stable iodine concentration can be assumed from the radioiodine concentrations. It has long been recognized that a barrier prevents the free passage of halides (except chloride) into the cerebral spinal fluid (CSF) (Weir and Hastings, 1939), which results in a low concentration of all halides relative to the chloride concentration (Weir and Hastings, 1939; Wallace and Brodie, 1937; 1939a; 1939b).

This results from brain tissue equilibration with the halides of the CSF, rather than serum halides.

Other tissues of the body seem to have a similar distribution of both iodide and chloride. Variations are introduced into the tissue/serum values because of differences in extracellular space, including vascularity, as well as by the unequal introduction of I^{131} radiohormones into different tissues, or the same tissue at different times (Eales, 1963). The ratio of I^{131} in red blood cells/I^{131} in plasma averaged 0.08 in this study, indicating low concentrations of I^{131} in the red blood cells. The ratio for salmon may reach this value, but Leloup and Fontaine (1960) have reported a red blood cell/plasma ratio of 0.23 for rainbow trout. The reason for this discrepancy is unknown. Red blood cells/plasma values for chloride and I^{127} were not obtained.

Blood analysis revealed only small amounts of iodinated tyrosines (PBI - BEI = 0.08 μg. %), suggesting that only small quantities of these compounds leave the thyroid. PBI is a poor estimate of circulating hormone levels in poikilotherms, since only a small fraction of the PBI is metabolically active hormone (BEI) (Hickman, 1962; Meisner, 1961). However, this may not be the case with trout under the conditions of this experiment. A large portion of the inorganic iodine was probably originally bound to plasma proteins, but this linkage is destroyed by protein precipitation.

Any analysis of the body tissue distribution of a radioisotope must be made under equilibrium conditions. In non-equilibrium conditions, thyroid uptake and excretion from the body increase individual variability, since loss of iodine-131 from body tissues depends directly on the rates

of excretion and uptake.

Use of specific activity for calculation of secretion rates and absolute tissue concentrations of stable iodine must be performed under steady state conditions, both for radio- and stable-iodine. Daily injections of radioiodine sufficient to restore initial body concentrations of the isotope are probably not frequent enough to maintain complete I^{131} equilibrium. This is most readily seen in the thyroidal tissue/ plasma ratio for total I^{131}. This value is over 21, while under equilibrium conditions it approximates 1.2-1.5. Addition of radioiodine to the water is preferable to injection, because the thyroid I^{131} concentration in the water changes only slowly. Equilibrium is attained rapidly from an environmental supply of radioiodine, an average thyroid T/S of 1.25 being reached in a matter of minutes.

In conclusion, the distribution of iodide is very similar to chloride distribution in muscle, gill, liver and spleen. Some degree of concentration of iodide, but not chloride, occurs in skin, kidney and carcass. Iodide is concentrated by the thyroid, and appears only in small amounts in brain tissue.

D. Iodine uptake from the water environment:

Large differences in the amounts of injected radioiodide accumulated by the thyroids of fish in iodine-rich and iodine-poor waters have been found (Gorbman, Berg & Creaser, 1953). Hickman (1959) found that thyroidal uptake of I^{131} by euryhaline flounder was depressed during adaptation to fresh water, if the water was fortified with amounts of iodine similar in concentration to that of normal sea water. Conversely,

flounder kept in iodine-deficient sea water had greater thyroidal accumulation of I^{131} than control fish maintained in normal sea water (see also Berg et al, 1959).

Depressed thyroid uptake in domesticated freshwater fish held in an environment richer in iodine than control concentrations show depressed thyroidal activity (Berg and Gorbman, 1953, 1954; Srivastava, 1960). The histological appearance of the gland is also one of decreased activity (Robertson & Chaney, 1953; LaRoche & Leblond, 1952). Consequently, thyroidal response in fish to iodine supply is similar to the thyroid response in mammals (LaRoche, 1950).

Depression of thyroidal I^{131} uptake occurred in the groups held in iodine-rich water (Fig. 1). The greater accumulation of I^{131} in the thyroids of those fish held in the iodine-poor water is a compensatory response to the low levels of iodine found in the water.

Thyroid uptake of I^{131} in rats and mice is depressed by starvation, due to decreased secretion of thyrotrophin (TSH) (Danowski, 1962; Mont, 1947). Depressed thyroid uptake has also been found in starved tadpoles, the depression increasing with time (Hunt and Dent, 1957), as well as in rainbow trout (Fontaine & Fontaine, 1956). Thyroid depression due to starvation can be seen in the thyroid uptake of starved fish in water not enriched with iodine (Fig. 1). Depression of thyroid activity does not occur in starved fish retained in iodine enriched water (Fig. 1). In addition, starved fish retain more stable iodine than do fed fish in iodine-enriched water (Fig. 4) (Table I). During partial or complete starvation, the rat thyroid is known to become more sensitive to TSH, although the circulating concentrations of TSH are decreased (Halmi, 1954; Mont, 1947). Increased

sensitivity to TSH during starvation has also been demonstrated in rainbow trout (Leloup & Fontaine, 1960), and is probably caused by low levels of organified iodine in the blood, as well as the direct regulatory effect that blood iodide levels have on the thyroid (Chapman, 1941; Galton & Pitt-Rivers, 1959; Mont, 1947; Wolff & Chaikoff, 1948), (also see Results, Section A). Trapping of iodide from iodine-rich water (even during starvation - see Table I), and decreased demand for thyroid hormone that accompanies reduced metabolic rate (if thyroxine exerts a calorigenic effect - Hickman, 1959; Olivereau & Francotte-Henry, 1956), when coupled with decreased fecal excretion of thyroxine (Intoccio & Van Middlesworth, 1958), may put these fish in positive iodine balance.

In conclusion, the material presented gives evidence that the potential major source of iodine for fresh water teleost fish is the iodine present in the water environment. Dietary sources play a secondary role.

E. Effects of environmental iodide on uptake of dietary I^{131}:

It has been discovered that fish held in iodine-rich water possess a quiescent thyroid gland (LaRoche and Leblond, 1952) and under these conditions radioiodide is excreted at increased rates. Opposite effects, i. e. increased thyroid uptake and decreased rate of excretion, are found in fish held in iodine-poor water (Berg and Gorbman, 1953; Leloup and Fontaine, 1960; Hickman, 1959). In Hyla versicolor tadpoles, characteristics of thyroid uptake in iodine-rich and iodine-poor water are similar to those of fish under similar conditions. However, the rate of excretion is slow (Money et al. , 1955) and is not affected by iodine concentrations of either food or water (Hunt and Dent, 1957).

Generally, these findings lead one to expect comparable behaviour of radioiodine administered via the gut. This is in fact the case. Loss of dietary I^{131} from the whole body is markedly influenced by environmental iodine concentration (Fig. 2). However, Fig. 2 represents more than simple absorption from the gut, followed by excretion from the body. Represented here is radioiodine loss across the gills and excretion via the kidneys, as well as some iodine retention by the thyroid gland.

Radio- and stable-iodine are metabolically identical within the limits of experimental error (Sacks, 1953), but the specific activity (ratio of I^{131}:I^{127}) of iodine in the iodine-rich group is much smaller than in either of the other groups. Consequently, the excretion of a given amount of I^{131} by the fish in iodine-rich water indicates the excretion of more I^{127} by these fish than by fish in a lower concentration of I^{127}. These same principles of specific activity apply to any consideration of the control and iodine-poor groups. Thus, these observations with radioiodine underestimate the rate of total iodide excretion, since the specific activities of the ingested radioiodide to water iodide are in the relationship of 1/23000:1/67;1/1 for iodide-rich:control:iodide-poor group, respectively. These observations agree with the conclusions of Leloup and Fontaine (1960) and Berg and Gorbman (1953) that iodine-rich water accelerates the rate of total excretion of iodine from the body.

In summary, the iodine concentration of the water has a profound and rapid influence on the conservation and utilization of dietary iodine. The degree of conservation and the speed of excretion vary with the iodine concentration of the medium.

F. Uptake of radioiodine by gill tissue:

The presence of large acidophil cells, commonly called "chloride secretory cells", has been noted in the vascular parts of the inner operculum and the pseudobranch, as well as in gill filaments (Burns & Copeland, 1950). These cells are known to transport chloride and other monovalent ions from the water (Krogh, 1937, 1939; Black, 1957).

Chloride cells could not be identified by the staining method employed and no localization of radioactivity could be found. Cytological identification of chloride cells requires more refined histological methods, such as mitochondrial stains or electron microscopy (Threadgold and Houston, 1961; Getman, 1950).

Although gill epithelial cells do not seem to actively transport iodide, failure of the pseudobranch to do so is not surprising. Pseudobranchs have long been assigned an osmoregulatory role (Burns & Copeland, 1950), despite the fact that they are often deeply buried in the sides of the head (Doyle & Gorecki, 1961). They may also be completely absent, as in the eel (Anguilla), the experimental fish used by Krogh (1937). Removal of the pseudobranchs from fish causes no osmoregulatory or respiratory distress (Parry, 1959). Glandular functions have been suggested for the pseudobranch (Copeland & Dalton, 1959; Parry & Holliday, 1960).

Radioautography, the method used in this investigation, has one serious shortcoming. If passage of radioiodine through the cells of the gills was accomplished rapidly enough, cellular accumulation of radioiodine would be minimal. However, by means of radioautographs, radioiodine transport has been demonstrated in thyroid follicular cells

by the visualization of radioiodide in the cells (Leblond & Gross, 1948), indicating the method has some merits.

Thyroid follicular cells transport iodide against a 25-fold concentration gradient in mammals. If iodide transport occurred through the gills into the body, this would have to occur against a gradient of from two to fifty in marine fish and up to a thousand in fresh water fish (Hickman, 1962; also see Results, part B). If the transport mechanism was present in the cells of the gills and transport here was at a rate similar to the rate of transfer found in thyroid follicular cells, then it should be evident by this technique.

Autoradiographic techniques have failed to demonstrate any active transport of iodide from the water environment. However, it is felt by the author, intuitively at least, that a more sophisticated method is desirable for a study of the transport of iodine by the respiratory epithelium. Perhaps a more profitable approach would be an investigation of the iodide secretory powers of the chloride cells, since thiocyanate, an ion similar to the halides in behaviour, is known to be secreted by gill and oral membranes of the dog fish, Squalus, (Thorson, 1958). Perfusion of a heart-gill preparation,(as used by Schiffman (1961)), with the radioisotope of iodine, is a possible approach. (The method was tried in this study, but the technical difficulties could not be overcome.) Subsequent poisoning with cyanide, or other methods of metabolic inhibition, should divorce the effects of simple diffusion from any active transport that might occur.

In summary, this approach failed to demonstrate any active iodide absorption from the environment by the cells of the respiratory and buccal

tissues. These results agree with the work of Krogh (1939) on goldfish and Maqsood et al. (1961) on rainbow trout.

G. Ingestion of water:

Information available concerning the amount of water imbibed by fresh water fish is contradictory (see literature review of Allee and Frank, 1948). Until the work of Allee and Frank (Allee and Frank, 1948; Frank and Allee, 1950), water was thought to enter the body osmotically across gill and oral membranes, since the body fluids of fresh water fish are hypertonic to the environment (fresh water: -0.03°C; teleost serum: -0.57°C), (Black, 1957; Pross and Brown, 1961). However, Allee and Frank (op.cit.) found that several species of fish, filter feeders as well as pisciverous species, did drink water.

From the results of this experiment and the findings of other workers, it can be concluded that water drinking in rainbow trout is minimal, and only a minute amount of iodine is gained from this source.

H. I^{131} metabolism by isolated fish skin in vitro:

The incorporation of radioiodide into melanin pigments, especially in the pigmented retina of the frog tadpole eye, has been reported (Dent and Hunt, 1952). Oxidation of iodide and the subsequent formation of mono- and di-iodotyrosine is known to occur in the skin of the adult frog, with larger concentrations of I^{131}-tyrosines occurring in the darkly pigmented dorsal skin than in the pale skin of the underside (Gennaro and Clements, 1956).

It is well established that the state of the thyroid gland has a definite effect on the melanin pigments in fish and frogs, since iodotyrosines are not used in melanogenesis (Dent and Hunt, 1952). Hyperthyroidism, or the administration of exogenous thyroxine, causes blanching in frogs and fish (Warren, 1940, cited by Dent and Hunt, 1952; LaRoche and Leblond, 1952) by decreasing the size and number of melanophores and obscuring more deeply buried melanophores by increasing the thickness of the skin. Antithyroid drugs also prevent melanogenesis in frog tadpoles (Dent and Hunt, 1952).

Consequently, it would appear that the oxidative enzymes that convert tyrosine to melanin facilitate union of iodine and tyrosine (Dent and Hunt, 1952). This tyrosine-melanin system seems to be sensitive to TSH, since conditions resulting in increased TSH secretion cause skin blanching, which may be partly due to enhanced formation of iodoamino acids.

Production of thyroid hormone by thyroidectomized and thyroidectomized-hypophysectomized rats supplied with an adequate amount of iodine was reported (Morton et al., 1943), but not confirmed (Taurog et al., 1960). However, it has been postulated that the calorigenic effect of large doses of stable iodide may be due to production of thyroxine-like materials in non-thyroidal tissues (Evans et al., 1960; Chapman, 1941).

The formation of thyroxine by certain invertebrates has been found by radioiodine methods (Gorbman, 1955; Gorbman et al., 1954). In addition, Gorbman concluded that thyroxine is easily formed in nature and that the occurrence of thyroxine may have preceded evolution of the

thyroid gland.

It would appear that extrathyroidal organification of iodide is not impossible and that biologically active thyroxine-like compounds can be produced in areas of melanin pigmentation, including the skin of fish.

I. Radioiodine contaminants:

The extraneous bands present in the radioiodide are presumably different oxidation forms of iodine, since the addition of cysteine, stable iodide and sodium thiosulphate converges these bands into the iodide-131 band (Dr. Fawcett, University of Alberta Hospital, personal communication, 1963; Doctor, 1960). One peak was probably the same compound denoted as U_1 by Taurog et al. (1957), since it failed to migrate from the line of sample application. The contaminating band of radioactivity that disappeared when incubated with the tissue may be U_2 of Taurog et al. (1957). This product has been formed by simple hydrogen peroxide oxidation of I^{131} (De Groot and Berger, 1960) as well as by oxidative enzymes (Taurog et al., 1957). It is quickly destroyed by mildly acid conditions and may have been reconverted to iodide-131 during extraction procedures.

The unknown compound (Rf = 0.80) remains unidentified.

Caution in the use of old supplies of I^{131} is advisable, since the various compounds that form during storage are not accumulated by the thyroid as rapidly as iodide (Doctor, 1960). Cysteine is normally present (0.2%) as a preservative, but great dilution seems to render it less effective, so addition of a reducing agent is recommended.

J. Secretion rate:

The secretion rate of hormonal iodine (μg /100 g body weight/ day) can be calculated by the use of formulae for turnover time, given by

Srivastava (1960): $T_t^1 = \frac{\log e \times t}{\log A_t - \log A_o}$

or Comar (1955), modified to the form: $T_t^1 = (2.3 \log \frac{(A_t)}{(A_o)} \frac{1}{t})^{-1}$

where T_t^1 = estimates of turnover time
A_t = activity at time t
A_o = activity at time o

The turnover time equation of Leloup and Fontaine (1960);

$T_t = \frac{\text{thyroid } I^{127}/100 \text{ g} \cdot \text{body weight}}{\text{secretion rate}}$

can be solved for secretion rate, using the estimates of turnover time derived above. Secretion rate of small control fish is approximately 2.6 µg. hormonal iodine/100 g body weight/day. Iodine-enriched water increases the secretion rate only slightly to 2.9 µg./100 g body weight/day.

Hoffert and Fromm (1959) determined the secretion rate of summer rainbow trout (13° C.) to be 0.3 µg. of thyroxine/100 g body weight/day (0.2 µg. of hormonal iodine). This agrees closely with Leloup and Fontaine (1960). The fish used by Hoffert and Fromm were much larger than the trout used in this laboratory (14 g compared to 0.53 (C) and 0.57 (IR) g in this study). Hormonal secretion rate is much higher in small fish (Eales, 1963), the rate of secretion decreasing greatly with increasing body size, as it does in mammals (Pitt-Rivers and Tata, 1959).

K. Ecological significance:

A point of considerable importance in a comparison of dietary and environmental iodine supplies is the periodicity of the dietary supply. Dietary iodine is subject to daily and seasonal fluctuations. Insectivorous

fish, like rainbow trout, feed most heavily at the twilight times of day; dawn and dusk. Consequently, dietary contribution to blood iodine has two main peaks during the day, following each feeding.

The seasonal variation in dietary iodine is even more pronounced. In regions where winters are cold, fish often undergo many months of starvation. During migration, steelhead and salmon endure long periods of fasting in fresh water. Their extreme muscular activity (often at fairly high temperatures) is known to increase iodine demands during these periods (Eales, 1963). Thus, the only iodine available to fish during long periods of starvation is contained in the water.

Another dietary consideration is the iodine content of the food. Food supplied in this laboratory is relatively rich in iodine, providing well over 20 times as much iodine to trout as would a diet composed of the whole bodies of their own kind. Any diet consisting of small cyprinids and minnows would probably supply even less iodine, since fish of this type often have sluggish thyroids. A diet composed of various insects is extremely low in iodine, since insects store no iodine and only minute amounts of iodine are present, principally incorporated in the chitin of the cuticle, which remain undigested.

Iodine available to fish as inorganic ions from the water has several advantages, despite the low concentration found in fresh water. The lack of any appreciable fluctuations in the iodine content of fresh water, particularly periods of great dilution, allows a steady rate of absorption. This inorganic iodine is available in a form readily used by the body. In addition, this iodine is continuously available with a minimal expenditure

of energy, whether by transport or diffusion.

Examination of Fig. 4 and Table I reveals that fed fish (C and IR) are in total equilibrium regardless of their environmental iodine concentration. On the other hand, starved fish (St) are in negative iodine balance, while starved fish held in iodine-enriched water (IR-St) are in positive balance. This suggests that at some iodine concentration between 0.1 and 30 ug. I^-/litre of water, iodine accumulation can become independent of dietary iodine. At this concentration, any fish, whether starved or fed, will be in total iodine equilibrium.

This study has raised several interesting questions, none of which have been resolved.

A clear similarity of distribution of iodide and chloride has been found in the body musculature of rainbow trout. The reduced concentration of iodide in muscle gives the whole body an iodide space of approximately 50%. However, the iodide space of starry flounder rapidly reaches 100%. Does this mean that skeletal muscle of starry flounder is equally permeable to chloride? Or, alternatively, is the behaviour of iodide and chloride different in flounder? Still another possibility may be the presence of extrathyroidal iodine depots in the bodies of marine flounder.

Another question can be asked on the basis of Leloup and Fontaine's (1960) report that starvation increases I^{131} uptake when TSH is administered to rainbow trout. Does this same pattern occur in starved fish held in iodine-enriched water? This present study suggests that it does not. Further work will be undertaken along this line.

SUMMARY

(1) Penetration of environmental iodide into the body is rapid. Equilibration of body I^{131} with body I^{127} occurs within minutes.

(2) Autoradiography failed to demonstrate active transport of iodide by the chloride secretory cells of the gills.

(3) The rate and degree of excretion of dietary radioiodine is affected directly by the stable iodine concentration of the environment.

(4) Thyroid uptake of environmental I^{131} varies inversely with the concentration of iodine-127 in the environment.

(5) Iodide concentrating ability of the thyroid seems to depend on binding (a thyroid "trap") rather than active iodide "pumping".

(6) Whole body concentrations of stable iodine vary directly with the concentration of I^{127} in the water.

(7) Starved fish in relatively poor iodine conditions are in negative iodine balance. Starved fish in iodine-enriched water are in positive iodine balance, possibly as a result of decreased thyroid hormone utilization and decreased fecal excretion.

(8) Iodine received from the small amounts of water imbibed by rainbow trout is of little importance as a source of iodine.

(9) Iodine metabolism occurs in isolated preparations of melanin pigmented skin. Three analogues of thyroxine - mono- and di-iodotyrosine, triiodothyronine - as well as thyroxine are produced.

(10) Generally, the distribution of iodide in the body is similar to that of chloride. Iodine is accumulated by the thyroid and appears at increased concentrations in the gut, kidney and skin. Iodine

accumulation in the brain is greatly reduced, probably because of low iodide concentrations in CSF.

(11) It is concluded that iodine derived from the water, at least in iodine-enriched water, is a more important source of iodine than is iodine derived from the food.

BIBLIOGRAPHY

1. Acland, J. D. 1957. The Estimation of Serum Protein-bound Iodine by Alkaline Incineration. Biochem. Jour. 66: 177-188.

2. Allee, W. C. and P. Frank. 1948. Ingestion of Colloidal Material and Water by Goldfish. Physiol. Zool. 21: 381-390.

3. Baumann, E. J. and N. Metzger. 1949. Behaviour of the Thyroid towards Elements of the Seventh Periodic Group. I. Halogens and Thiocyanates. Proc. Soc. Exp. Biol. & Med. 70: 536-540.

4. Baumann, E. J., N. Searle, A. A. Yalow, E. Siegel and S. M. Seidlin. 1953. Filtration of Seventh Group Elements by the Thyroid Studied with Radioactive Isotopes. "Abstracts of Communications" XIX International Physiol. Cong., Montreal, 1953.

5. Baumann, N. J., N. Zizmer, E. Oshry and S. M. Seidlin. 1949. Behaviour of the Thyroid Towards Elements of the Seventh Periodic Group. II. Rhenium. Proc. Soc. Exp. Biol. & Med. 72: 502-506.

6. Berg, O. and A. Gorbman. 1953. Utilization of Iodine by the Thyroid of the Platyfish, Xiphophophorus (Platypoecilus) maculatus. Proc. Soc. Exp. Biol. & Med. 83: 751-756.

7. Berg, O. A. and A. Gorbman. 1954. Normal and Altered Thyroidal Function in Domesticated Goldfish, Carassius auratus. Proc. Soc. Exp. Biol. & Med. 86: 156-159.

8. Berg, O., A. Gorbman and H. Kobayashi. 1958. The Thyroid Hormones in Invertebrates and Lower Vertebrates. Comparative Endocrin., Ed. by A. Gorbman. Wiley & Sons, N.Y., p. 302-319.

9. Black, V. S. 1957. Excretion and Osmoregulation. Physiol. of Fishes, Vol. 1. (Ed.) M. E. Brown, Academic Press, N.Y.

10. Block, J. R., E. L. Durrum and G. Zweig. 1958. A Manual of Paper Chromatography and Paper Electrophoresis. Academic Press, N.Y.

11. Bois, I. and L. G. Larsson. 1958. Effect of Varying Iodine Supply on Labelled Iodine Fractions in the Thyroid Gland After I^{131} Administration. Acta Endocrin., 28: 262-272.

12. Burns, J. and D. G. Copeland. 1950. Chloride Excretion in the Head Region of Fundulus heteroclitus. Biol. Bull. 99: 381-385.

13. Chapman, A. 1941. Extrathyroidal Iodine Metabolism. Endocrin. 29: 686-694.

14. Chapman, A. 1941. The Relation of the Thyroid and the Pituitary Glands in Iodine Metabolism. Endocrin. 29: 680-685.

15. Comar, C. L. 1955. Radioisotopes in Biology and Agriculture; Principles and Practice. McGraw-Hill, N. Y., Toronto, London.

16. Copeland, D. E. and A. J. Dalton, 1959. An Association between Mitochondria and the Endoplasmic Reticulum in Cells of the Pseudobranch Gland of a Teleost. Jour. Biophys. & Biochem. Cytol. 5: 393-396.

17. Cotlove, E. 1963. Determination of the True Chloride Content of Biological Fluids and Tissues. II. Analysis by Simple Nonisotopic Methods. Analytical Chem. 35 (1): 101-105.

18. Danowski, T. S. 1962. Clinical Endocrin. Vol. II: Thyroid. The Williams and Wilkins Co., Baltimore. (540 pp.)

19. DeGroot, L. J. and J. E. Berger. 1960. A Note on Inorganic Oxidation Products of I^{131} Formed in Biological Systems. Endocrin. 67: 657-662.

20. Dent, J. N. and E. L. Hunt. 1952. An Autoradiographic Study of Iodine Distribution in Larvae and Metamorphosing Specimens of Anura. Jour. Exp. Zool. 121: 79-97.

21. Doctor, V. M. 1960. Effect of Storage of NaI^{131} Solutions upon the Thyroid Uptake by Day-Old Chicks. Endocrin. 66: 559-564.

22. Doyle, W. L. and D. Gorecki. 1961. The So-called Chloride Cell of the Fish Gill. Physiol. Zool. 34: 81-85.

23. Eales, J. G. 1963. An Analysis of the Thyroid Role in Juvenile Steelhead (Salmo gairdneri Richardson) and Factors Responsible for its Seasonal Fluctuation in Activity. Ph. D. Thesis, U. B. C.

24. Evans, E. S., A. Taurog, A. A. Koneff, G. D. Potter, I. L. Chaikoff and Simpson. 1960. Growth response of Thyroidectomized Rats to High Levels of Iodide. Endocrin. 67: 619-634.

25. Fontaine, M. and Y. A. Fontaine. 1956. Determination du pouvoir thyreotrope de l'hypophyse et du milieu interieur de teleosteens par mesure de la fixation de 131-I par la thyroide de la truite arc-en-ciel (Salmo Gairdneri). Jour. Physiologie (Paris) 48: 881-892.

26. Frank, P. and W. C. Allee. 1950. Ingestion of Colloidal Thorium Dioxide by Representative Minnows from the Chicago Region. Physiol. Zool. 23: 134-139.

27. Galton, V. A. and R. Pitt-Rivers. 1959. Effect of Excessive Iodide on the Thyroid of the Rat. Endocrin. 64: 835-837.

28. Gennaro, J. F. and M. M. Clements. 1956. The Incorporation of I^{131} in the Frog Skin in vitro and in vivo. Anat. Rec.124: 294 (Abst.)

29. Getman, H. C. 1950. Adaptive Changes in the Chloride Cells of Anguilla rostrata. Biol. Bull. 99: 439-445.

30. Gorbman, A. 1955. Some Aspects of the Comparative Biochemistry of Iodine Utilization and the Evolution of Thyroidal Function. Physiol. Rev. 35: 336-346.

31. Gorbman, A. and O. Berg. 1955. Thyroidal Function in the Fishes Fundulus heteroclitus, Fundulus majalis and Fundulus diaphanus. Endocrin. 56: 86-92.

32. Gorbman, A., O. Berg and C. W. Creaser. 1953. Thyroidal Iodine Accumulation of Several Species of Fresh and Brackish Water Teleost Fishes. Anat. Rec. 117: 535

33. Gorbman, A. and H. A. Bern. 1962. A Textbook of Comparative Endocrinology. Wiley Co., N.Y.

34. Gorbman, A., M. Clements and R. O'Brien. 1954. Utilization of Radioiodine by Invertebrates, with Special Study of Several Annelida and Mollusca. J. Exp. Zool. 127: 75-89.

35. Gross, J. 1962. Iodine and Bromine in Mineral Metabolism. Vol. II: Part B. Ed. by C. L. Comar and F. Bronner. Academic Press, N. Y. and London.

36. Hale, H. J. 1958. Biological Laboratory Data. Methuen Monograph: Methuen and Co., London.

37. Halmi, N. S. 1954. Regulation of the Rat Thyroid in Short-Term Iodine Deficiency. Endocrin. 54: 216-224.

38. Halmi, N. S., B. N. Spirtos, E. M. Bobdanove, and H. J. Lipner. 1953. A Study of Various Influences on the Iodine Concentrating Mechanism of the Rat Thyroid. Endocrin. 52: 19-32.

39. Hickman, C. P. Jr. 1959. The Osmoregulatory Role of the Thyroid Gland in the Starry Flounder, Platichthys stellatus. Can. J. Zool. 37: 997-1060.

40. Hickman, C. P. Jr. 1962. Influence of Environment on the Metabolism of Iodine in Fish. Gen. & Comp. Physiol., Supplement 1: 48-62.

41. Hoffert, J. and P. O. Fromm. 1959. Estimation of Thyroidal Secretion Rate with I^{131}. Jour. Cell. Comp. Physiol. 54: 163-169.

42. Hunt, E. L. and J. N. Dent, 1957. Iodine Uptake and Turnover in the Frog Tadpole. Physiol. Zool. 30: 87-91.

43. Intoccio, A. and L. Van Middlesworth. 1958. Thyroxine Excretion Increase by Cold Exposure. Endocrin. 64: 462-464.

44. Isler, H., C. P. Leblond and A. A. Axelrad. 1958. Mechanism of the Thyroid Stimulation Produced by Sodium Chloride in the Mouse. Endocrin. 62: 159-172.

45. Krogh, A. 1937. Osmotic Regulation in Fresh Water Fishes by Active Absorption of Chloride Ions. Zeit. Vergl. Physiol. 24: 656-666.

46. Krogh, A. 1939. Osmotic Regulation in Aquatic Animals. Cambridge Univ. Press. 242 pp.

47. LaRoche, G. 1950. Fixation du radioiodide par la gland thyroide du saumon de l'Atlantique. Annales de l' A.C.F.A.S. 16: 134-137.

48. LaRoche, G. and C. P. Leblond. 1952. The Effect of Iodide and Thyroid Preparations on Salmonidae. Endocrin. 51: 524-545.

49. Leblond, C. P. and J. Gross. 1948. Thyroglobulin Formation in the Thyroid Follicle Visualized by the "Coated Autograph" Technique. Endocrin. 43: 306-324.

50. Leloup, J. and M. Fontaine. 1960. Iodine Metabolism in Lower Vertebrates. Ann. N.Y. Acad. Sc. 86: Art. 2. 316-351.

51. Lipner, H. and S. Hazen. 1962. Extrathyroidal Iodine Pump in Tadpoles (Rana grylio). Science 138 (#3543): 898-899.

52. Magsood, M., E. P. Reineke, and P. O. Fromm. 1961. In vitro I^{131} Uptake by Tissues of Rainbow Trout. Physiol. Zool.:34: 34-38.

53. Meisner, H. M. 1961. The Effect of Propylthiouracil on the Oxygen Consumption and Radioiodine Metabolism of the Frog Rana pipiens in Winter. M. Sc. Thesis, Univ. of Alberta.

54. Money, W. L., V. Lucas and R. W. Rawson. 1955. The Turnover of Radioiodine (I^{131}) by the Rana pipiens Tadpole. Jour. Exp. Zool. 128: 411-422.

55. Mont, F. G., K. E. Paschkis and A. Cantarow. 1947. Thiouracil Action on Starved Rats. Endocrin. 40: 225-26.

56. Morton, M. E., I. L. Chaikoff, W. O. Reinnardt and E. Anderson. 1943. Radioactive Iodine as an Indicator of the Metabolism of Iodine VI. The Formation of Thyroxine and Diiodotyrosine by theCompletely Thyroidectomized Animal. J. Biol. Chem. 147: 757-769.

57. Olivereau, M. and M. Francotte-Henry. 1956. Etude histologique et biometrique de la glande thyroide de Caecobarbus geertsi. Ann. Soc. Zool. Belg. 86: 129-150. cited by Hickman, 1959.

58. Parry, G. 1959. Chloride Secretory Cells in Gills of Teleosts. Nature 183: 1248-1249.

59. Parry, G. and F. G. T. Holliday. 1960. An Experimental Analysis of the Function of the Pseudobranch in Teleosts. Jour. Exp. Biol. 37: 344-354.

60. Pastan, I. 1957. Absorption and Secretion of Iodine by the Intestine of the Rat. Endocrin. 61: 93-97.

61. Pitt-Rivers, R. and J. R. Tata. 1959. The Thyroid Hormones. Pergamon Press, Los Angeles.

62. Prosser, C. L. and F. A. Brown. 1961. Comparative Animal Physiology. W. B. Saunders Co., Philadelphia.

63. Riggs, D. S. 1952. Quantitative Aspects of Iodine Metabolism in Man. Pharmacol. Rev. 4: 284-370.

64. Robertson, O. H. and A. L. Chaney. 1953. Thyroid Hyperplasia and Tissue Iodine Content in Spawning Rainbow Trout: A Comparative Study of Lake Michigan and California Searun Trout. Physiol. Zool. 26: 328-340.

65. Sacks, J. 1953. Isotopic Tracers in Biochemistry and Physiology. McGraw-Hill Book Co. Inc., N.Y., London, Toronto.

66. Sasaki, T. and H. Nakajima. 1962. Effects on the Thyroid in Young Rats of Excessive Fecal Thyroxine Excretion. Endocrin. 71: 520-522.

67. Schiffman, R. H. 1961. A Perfusion Study of the Movement of Strontium Across the Gills of Rainbow Trout. (Salmo gairdneri). Biol. Bull. 120 (1): 110-117.

68. Slingerland, D. W. 1955. The Influence of Various Factors on the Uptake of Iodine by the Thyroid. Jour. Clin. Endocrin. and Metab. XV: 131-141.

69. Srivastava, P. N. 1960. Utilization of Radioiodine by the Thyroid Gland of Goldfish. (Carassius auratus L.) Physiol. Zool. 33: 277-280.

70. Taurog, A., E. S. Evans, G. D. Potter and I. L. Chaikoff. 1960. Failure to Demonstrate Extrathyroidal Thyroxine Formation in Thyroidectomized Rats on Low and Moderate Iodine Intakes. Endocrin. 67: 635-649.

71. Taurog, A., W. Tong and I. J. Chaikoff. 1957. An Unidentified Iodine Compound Formed by Incubation of Cell-Free Preparations of Tissue with Iodide - I^{131}. Jour. Biol. Chem. 227: 759-772.

72. Thorson, T. B. 1958. Measurement of the Fluid Compartments of Four Species of Marine Chondrichthyes. Physiol. Zool. 31: 16-23.

73. Threadgold, L. T. and A. H. Houston. 1961. An Electron Microscope Study of the 'Chloride Secretory Cell' of Salmo salar L., with Reference to Plasma Electrolyte Regulation. Nature 190:612-614.

74. Van Middlesworth, L. 1956. A Method for Iodide Balance Studies in Animals on Low Iodide Diets. Endocrin. 56: 235-242.

75. Vinogradov, A. P. 1953. The Elementary Chemical Composition of Marine Organisms. Sears Found. Mar. Res. Mem. #11.

76. Wallace, G. B. and B. B. Brodie. 1937. Distribution of Administered Iodide and Thiocyanate in Comparison with Chloride and Their Relation to the Body Fluids. Jour. Pharmacol. Exp. Therap. 61: 397-406.

77. Wallace, G. B. and B. B. Brodie, 1939. The Distribution of Administered Bromine inComparison with Chloride and its Relation to Body Fluids. Jour. Pharmacol. Exp. Therap. 65: 214-219.

78. Wallace, G. B. and B. B. Brodie. 1939. The Distribution of Iodide, Thiocyanate, Bromide, and Chloride in the Central Nervous System. Jour. Pharmacol. Exp. Therap. 65: 220-226.

79. Weir, E. G. and A. B. Hastings. 1939. The Distribution of Bromide and Chloride in Tissues and Body Fluids. Jour. Biol. Chem. 129: 547-558.

80. Wolff, J. and I. L. Chaikoff. 1948. Plasma Inorganic Iodide, a Chemical Regulator of Normal Thyroid Function. Endocrin. 42: 468-471.

81. Yagi, Y., R. Michel and J. Roche. 1953. Bull. Soc. Chim. Biol. Paris 35: 289. Cited in Pitt-Rivers and Tata, 1959.

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